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Antikaliuretic action of trimethoprim is minimized by raising urine pH

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Antikaliuretic action of trimethoprim is minimized by raising urine pH. This study was designed to test the hypothesis that the antikaliuresis caused by trimethoprim could be diminished by alkalinizing the luminal fluid in the CCD, thereby converting trimethoprim from its cationic, active form to an electroneutral, inactive form. Timethoprim-induced inhibition of transepithelial Na^+ transport was examined in A6 distal nephron cells by analysis of short circuit current. The voltage-dependence of the trimethoprim-induced block of Na^+ channels was examined with patch clamp recordings of A6 cells. The antikaliuretic effect of trimethoprim was examined *in vivo* in rats pretreated with desoxycorticosterone and with NH_4Cl to lower urine pH, and in rats also receiving acetazolamide to raise urine pH. We found that the concentration of trimethoprim required to inhibit the amiloride sensitive component of short circuit current by 50% (IC_{50}) was 340 μM (at pH 8.2) and 50 μM (at pH 6.3). The IC_{50}s of protonated trimethoprim were similar (34 μM at pH 8.2 and 45 μM at pH 6.3). The mean time open for the high selectivity, Na^+ channel was reduced from 1679 ± 387 msec to 502 ± 98 msec with addition of 10^{-5} M trimethoprim to patch pipette solution at the resting membrane potential ($-V_{pipette}$ = 0 mV). Further decreases in mean time open were observed as $-V_{pipette}$ was reduced (that is, apical membrane hyperpolarization) to −40 mV (mean time open = 217 ± 85 msec) and to −80 mV (mean time open = 69 ± 13 msec). *In vivo*, trimethoprim caused a > 50% reduction in potassium (K^+) excretion due primarily to a fall in the $[K^+]$ in the lumen of the terminal CCD. This effect of trimethoprim was markedly attenuated in an alkaline urine induced by acetazolamide. We conclude that it is the charged, protonated species of trimethoprim which blocks epithelial Na^+ channels. Increasing urinary pH decreases the concentration of the charged species of trimethoprim and minimizes its antikaliuretic effect.

Drugs used in the treatment of *Pneumocystis carinii* pneumonia (PCP) such as trimethoprim and pentamidine inhibit epithelial sodium (Na^+) channels present in renal cortical collecting ducts (CCD) [1–3]. Hence these drugs act in a similar fashion to potassium (K^+) sparing diuretics, and their administration to patients with the acquired immunodeficiency syndrome (AIDS) likely contributes to the high incidence of hyperkalemia observed in patients with AIDS who receive these antimicrobial agents [4–6].

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Amiloride is the prototypic epithelial Na^+ channel inhibitor [7, 8]; it is a weak base, and its protonated species is the form that inhibits epithelial Na^+ channels by mechanisms which are voltage dependent [8–12]. Trimethoprim is also a weak base with a pK_a of 7.24 (Table 1) [13]. We reasoned that it is likely that the protonated species of trimethoprim should be the form of the drug that will block epithelial Na^+ channels.

The purpose of the present study was to determine if the tendency of trimethoprim to inhibit renal K^+ excretion could be abrogated by converting trimethoprim to an inactive, electroneutral form in the lumen of the CCD. We speculated that when the urine pH is above 7, the cationic form of trimethoprim will decline appreciably. This could have a profound effect on trimethoprim's ability to block epithelial Na^+ channels, and thereby its secondary inhibitory effect on renal K^+ secretion. In the results to follow, we provide direct evidence that it is the protonated species of trimethoprim that blocks epithelial Na^+ channels in a voltage-dependent manner. Moreover, we demonstrate that the antikaliuretic effect of trimethoprim was due to a low luminal concentration of K^+ in the CCD ($[K^+]_{CCD}$). The low K^+ excretion rates attributed to trimethoprim were largely prevented by raising urine pH. These data suggest that urine alkalization may ameliorate trimethoprim-induced hyperkalemia in humans.

Methods

Chemicals

Trimethoprim was purchased from Sigma Chemical Corporation (St. Louis, MO, USA); it was diluted initially in DMSO as a stock solution which was diluted further in Ringer's solution in individual experiments. Desoxycorticosterone pivalate (DOCP) was purchased from Ciba-Geigy, Animal Health; acetazolamide was purchased from Cyanamid Canada Inc. (Montreal, Quebec, Canada). Appropriate solvent vehicles were of highest purity and, when added to the control bath by themselves, caused no change in Na^+ channel activity or short circuit current (I_{sc}).

A6 distal nephron cell line cultures

The methods are similar to those described previously [1–3, 14, 15]. Briefly, A6 cells derived from *Xenopus laevis* kidney (American Type Culture Collection, Rockville, MD, USA) or the A6 subclone 2F3 (a gift from Drs. B.C. Rossier and J.P. Kreahenbuhl) were maintained in plastic tissue culture flasks at 28°C with 4%

Table 1. pK_as of epithelial Na^+ channel blockers

Drug	pK_a
Triamterene [7]	6.5
Trimethoprim [13]	7.24
Amiloride [7, 8]	8.7
Pentamidine[a]	11.4

[a] Unpublished observations, Fujisawa, Deerfield, IL, USA

CO_2 in air as previously described [3]. A6 cells passages 71 to 85 were used for patch clamp experiments and the A6 subclone 2F3, passages 91 to 96, for transepithelial measurements. For patch clamp experiments, A6 cells were plated at confluent density on permeable, glutaraldehyde-fixed, collagen-coated Millipore-CM filters (Millipore Corp., MA, USA) attached to the bottoms of small lucite rings [2, 3, 14–16]. For transepithelial experiments, A6 cells were seeded on collagen-coated polycarbonate filters (Costar, CA, USA) at a density of 0.5 to 1.0 $\times$ 10^6 cells/cm^2 [1–3].

Transepithelial measurements

A6 cell monolayers were transferred to a modified Ussing chamber and bathed in a Ringer's saline containing (in mM): 100 NaCl, 4 KCl, 2.5 $NaHCO_3$, 1 KPO_4, 1 $CaCl_2$, 11 glucose, and buffered with either 10 MES (pH 6.3); or 10 Tris (pH 8.2) [1–3]. Electrical measurements were performed with a DVC-1000 voltage clamp (World Precision Instruments, FL, USA). The I_{sc} was allowed to stabilize before the addition of drug. The amiloride-sensitive component of the short circuit current was determined by adding 10^{-5} M amiloride to the luminal solution at the end of each experiment.

Patch clamp recording and analysis

Patch pipette and extracellular bath solutions consisted of a physiologic amphibian saline containing (in mM): 95 NaCl, 3.4 KCl, 0.8 $CaCl_2$, 0.8 $MgCl_2$, and 10 HEPES (pH 7.4). Experiments were performed at room temperature. Unitary channel events were measured and analyzed as previously described [2, 3, 14–16]. The convention for applied voltage to the apical membrane patch ($-V_{pipette}$) represents the voltage deflection from the patch potential (that is, negative voltage = hyperpolarization; positive voltage = depolarization of the resting membrane potential for cell-attached patches). Inward current (pipette to cell) is represented as downward transitions in single channel records. We used NPo (number of channels times the open probability) as a measure of channel activity, and mean time open ($<t_{open}>$) as a measure of open state kinetics [2, 3, 14–16]. Mean time open for N observed channels was calculated as follows:

$$<t_{open}> = \frac{(T) \times (NPo)}{n/2}$$

where n is the total number of transitions between states during the total recording period T, and NPo is calculated as previously described [2, 3, 14–16].

In vivo experiments

Male Sprague-Dawley rats (weight 350 to 550 g) were obtained from Harlan Sprague Dawley, Inc. (Indianapolis, IN, USA). To achieve bicarbonate-poor urine, rats received NH_4Cl, 300 mM in 5% (wt/vol) sucrose, in their drinking water for 24 hours prior to the studies; the mean intake was 18 $\pm$ 2.5 ml per day. To achieve a reasonable urine flow rate, all rats were given 25 ml of 0.9% (wt/vol) NaCl per kg, intraperitoneally at the start of the experiment. On the morning of the experiments, rats were given a single intramuscular injection of 5 mg DOCP per kg body wt to ensure that mineralocorticoids were not a limiting factor. Following these preparations, each rat was placed in an individual metabolic cage and urine collected every two hours by spontaneous voiding. The cages were sprayed with silicone to facilitate rapid and complete collections of urine. An arterial blood sample was also collected following the final urine collection.

Studies

There were four groups of rats: (1) six rats received no additional treatment; (2) 15 rats received trimethoprim (20 mg/kg) as a single intraperitoneal injection as their sole treatment; the trimethoprim was diluted in sterile water to a concentration of 10 mg/ml. To induce an alkaline urine pH, two additional groups of rats were studied: (3) 12 rats received acetazolamide (5 mg/kg) by intraperitoneal injection in addition to the same dose of trimethoprim given to group 2. (4) Finally, in group 4, eight rats received acetazolamide alone at the above dose.

Analytical techniques

The concentrations of Na^+ and K^+ in plasma and urine were determined by flame photometry (Radiometer, Copenhagen, Denmark); chloride was measured by electromimetic titration (Radiometer). Urine and plasma osmolality were measured as previously described [17]. Urine pH, plasma pH and P_{CO2} in arterial blood, were measured using a Radiometer blood gas analyzer as previously described [17].

Calculations

The transtubular potassium gradient (TTKG), a reflection of the driving force for net K^+ secretion in the CCD was calculated using the formula: $TTKG = (U/P)_K/(U/P)_{osm}$, as previously described [18, 19].

Statistics

Mean time open is reported as $\pm$ one SD. ANOVA was used for multiple comparisons [2, 3, 14, 15]. Results were considered significant if $P < 0.05$. Statistics were performed with SigmaStat (Jandel Scientific, San Rafael, CA, USA). For the *in vivo* studies, results for the four groups were first compared by one-way ANOVA. Results were considered significant if $P < 0.05$. For parameters where ANOVA indicated a significant value, three comparisons using Student's *t*-test were carried out: control versus trimethoprim alone; trimethoprim versus trimethoprim plus acetazolamide (to assess the effects of acetazolamide on trimethoprim treated rats); and acetazolamide alone versus acetazolamide plus trimethoprim (to assess the effects of trimethoprim on acetazolamide treated rats). The level of significance was adjusted to 0.017 (0.05/3, the number of comparisons, the Bonferroni correction).

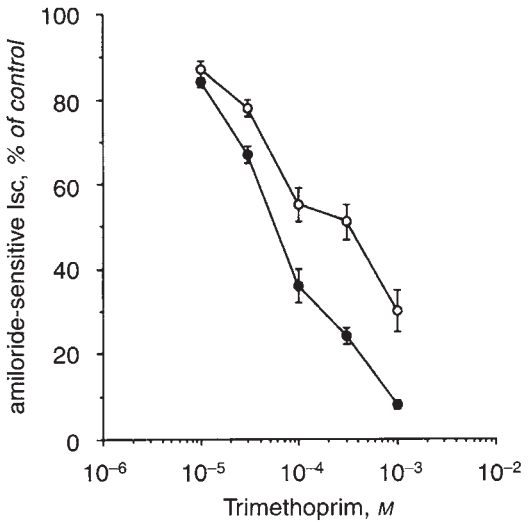

Fig. 1. *Effect of trimethoprim on transepithelial Na^+ transport (I_{sc}) in A6 distal nephron cells.* Results are expressed as percent inhibition of the amiloride-sensitive component of short circuit (I_{sc}) in A6 cells bathed in solutions buffered at pH 6.3 (●) or pH 8.2 (○). The results are the mean (± SEM) values from 4 experiments (pH 6.3) or 6 experiments (pH 8.2).

Results

Trimethoprim block of high selectivity Na^+ channels is pH-dependent

To examine whether it is the protonated form of trimethoprim that blocks Na^+ channels, the pH of the solution bathing the A6 cells was adjusted to either pH 6.3 or 8.2 in order to change the relative amounts of protonated trimethoprim (Fig. 1). The concentration of trimethoprim required to inhibit the amiloride sensitive component of I_{sc} (such as transepithelial Na^+ transport) by 50% (IC_{50}) at pH 8.2 was 340 μM ($N = 6$); the IC_{50} at pH of 6.3 was 50 μM ($N = 4$). We previously reported an IC_{50} of 120 μM at pH 7.4 [1]. These data were used to calculate the concentrations of protonated trimethoprim which inhibited the amiloride-sensitive component of I_{sc} by 50%; these concentrations were similar (between 34 and 49 μM, Table 2) at the three different pH values.

Trimethoprim block of high selectivity Na^+ channels is voltage-dependent

In cell-attached patches on the apical membrane of A6 cells, high selectivity Na^+ channels have all the characteristics of mineralocorticoid-sensitive, amiloride-blockable, Na^+ channels found in the apical membrane of principal cells of the mammalian CCD [2, 3, 14–16]. Under normal conditions (resting membrane potential, $-V_{pipette} = 0$ mV, room temperature, physiologic extracellular ion composition, and intact cell-attached configuration) the mean time open for the high sensitivity Na^+ channel was 1679 ± 387 msec ($N = 8$). This is consistent with basal mean time open values that we have measured previously [14]. Cell-attached patches were then made with patch pipettes containing the usual pipette saline plus 10^{-5} M trimethoprim. In A6 cells, we have previously shown that this clinically-relevant, "urinary" concentration of trimethoprim reduced Na^+ channel NPo by 83% [3]. At resting membrane potential ($-V_{pipette} = 0$ mV), we again found that exposure of the patch membrane surface to intrapipette trimethoprim significantly blocked apical Na^+ channel activity. Mean time open was reduced to 502 ± 98 msec ($N = 12$). In the presence of intrapipette trimethoprim, an additional decrease in mean time open was observed as $-V_{pipette}$ was made increasingly more negative (that is, apical membrane hyperpolarization; Fig. 2). At $-V_{pipette} = -40$ mV, mean time open fell to 217 ± 85 msec and at $-V_{pipette} = -80$ mV, the mean time open was only 69 ± 13 msec ($N = 12$). These data suggest that it is the charged, protonated species of trimethoprim which blocks epithelial Na^+ channels.

Table 2. Effects of pH on the inhibition of amiloride-sensitive short circuit current by trimethoprim (TMP) and by protonated trimethoprim (TMP^+)

Buffer pH	IC_{50} (TMP)	IC_{50} (TMP^+)
8.2	340	34
7.4	120	49
6.3	50	45

The IC_{50}s obtained with varying buffer pH are listed. The concentration of trimethoprim was determined by the Henderson-Hasselbalch equation.

In vivo studies

Rats fed NH_4Cl for one day prior to the experiment developed a mild rise in their plasma $[H^+]$ (fall in the plasma pH), a small fall in their plasma bicarbonate concentration, and their urine pH was consistently below 6 (Table 3). Treatment with acetazolamide alone led to a statistically significant, greater fall in the plasma bicarbonate concentration than in the group treated with trimethoprim plus acetazolamide. The plasma K^+ concentrations were somewhat lower in the experimental groups than in the control rats.

The key data are the urinary parameters. Urine flow rates were not statistically different between the four groups by ANOVA ($P > 0.05$). The urine K^+ concentration did differ between the four groups; rats treated with trimethoprim alone had a significant fall in the urine K^+ concentration compared to control, and this effect was significantly blunted when acetazolamide was given together with trimethoprim. Similarly, the urine K concentration was significantly lower in the group given trimethoprim with acetazolamide than with acetazolamide alone. Results for urine K excretion rate were parallel. Trimethoprim caused an approximately 50% fall in K excretion in both control and acetazolamide treated rats; acetazolamide doubled K excretion in trimethoprim treated rats.

The basis for changes in K excretion was analyzed using the TTKG. The TTKG was diminished by close to 50% in trimethoprim treated rats (3.7 ± 0.4) compared to controls (7.0 ± 0.4). In rats treated with both trimethoprim and acetazolamide, the TTKG was close to control (7.8 ± 0.8) and with acetazolamide alone, the TTKG was even higher than control (11.2 ± 0.7). Therefore, the changes in TTKG appeared to largely account for the changes in urinary K excretion rate.

Urinary Na^+ excretion differed between the groups, but the only statistically significant difference in the comparisons made was between trimethoprim alone versus trimethoprim plus acetazolamide. Acetazolamide caused a large natriuresis.

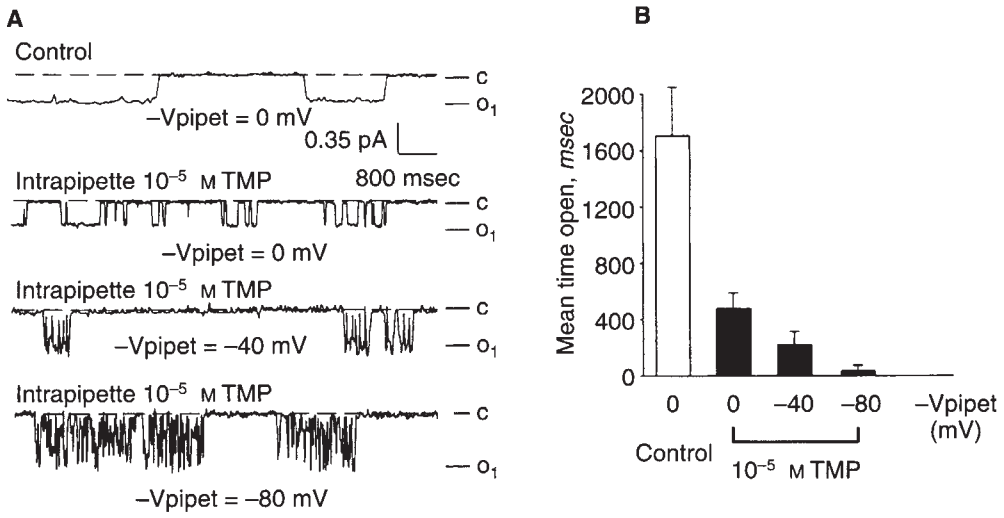

Fig. 2. *High selectivity, Na^+ channel block by trimethoprim.* (**A**) Single channel recordings from cell-attached patches containing only one Na^+ channel. At resting membrane potential ($-V_{pipette}$ = 0 mV), the control trace shows basal activity with typically long mean open times (753 ± 229 msec). In another patch, channel block was observed with exposure to intrapipette 10^{-5} M trimethoprim at $-V_{pipette}$ = 0 mV. In the same patch, rapid "flickering" between the open and closed states appeared when the apical membrane was hyperpolarized by 40 and 80 mV. Corner frequency was 1 Khz, sampling was performed at 2 Khz, and additional software filtering was 200 Hz. "C" marks the closed state and "O_1" marks the current level with one Na^+ channel open. (**B**) Plot depicts mean time open ($<t_{open}>$) in cell-attached patches containing multiple Na^+ channels. Control patches (N = 8) are plotted on the left and patches exposed to intrapipette 10^{-5} M trimethoprim (N = 12) are plotted on the right. Enhanced trimethoprim-induced blockade was observed with hyperpolarization from $-V_{pipette}$ of 0 mV to −80 mV.

Table 3. Effect of trimethoprim and acetazolamide on renal K^+ secretion

	Control (N = 6)	TMP (N = 15)	TMP + ACZ (N = 12)	ACZ (N = 8)
$[H^+]$ plasma *nM*	48 ± 1.8	43 ± 1.1	47 ± 2.1	49 ± 1.4
$[HCO_3]$ plasma *mM*	22 ± 1.1	24 ± 1.1	20 ± 0.8[b]	16 ± 0.8[c]
[K] plasma *mM*	4.3 ± 0.2	3.4 ± 0.1[a]	3.6 ± 0.1	3.6 ± 0.1
Flow rate *μl/min/kg body wt*	19 ± 2.7	22 ± 5.8	34 ± 9.9	36 ± 6.6
$[K]_{urine}$ *mM*	211 ± 18	78 ± 11[a]	144 ± 19[b]	224 ± 37
U_KV *nmol/min/kg body wt*	3784 ± 430	1525 ± 330[a]	3486 ± 623[b]	7129 ± 414[c]
U_{pH}	<6	6.0 ± 0.1	7.8 ± 0.1[b]	7.8 ± 0.1
TTKG	7.0 ± 0.4	3.7 ± 0.4[a]	7.8 ± 0.8[b]	11.2 ± 0.7[c]
$U_{Na}V$ *nmol/min/kg body wt*	3318 ± 547	2121 ± 548	7311 ± 1667[b]	8064 ± 981

Male Sprague-Dawley rats received NH_4Cl in their drinking water for 24 hours prior to the studies, DOCP on the morning of the experiment, and intraperitoneal 0.9% NaCl at the start of the experiment as described under **Methods**. Four groups of rats were studied: (1) no additional treatment; (2) trimethoprim (TMP) alone; (3) trimethoprim plus acetazolamide (ACZ); and (4) acetazolamide alone. The results are reported as the mean ± SEM; the number of rats is shown in parentheses. For details concerning statistics, see **Methods.**

[a] $P < 0.017$ for the effect of TMP as compared to normal rats.
[b] $P < 0.017$ for the effect of ACZ in TMP treated rats
[c] $P < 0.017$ for the effect of TMP in ACZ treated rats

Discussion

Transepithelial Na^+ transport across high resistance, Na^+ transporting epithelia is mediated by an amiloride-sensitive Na^+ channel in the apical plasma membrane and a Na^+,K^+-ATPase which is localized to the basolateral plasma membrane. Inhibition of epithelial Na^+ channels in the distal nephron secondarily inhibits K^+ secretion [7]. Previous studies of the amiloride-induced block of epithelial Na^+ channels have shown that it is the charged, protonated species which blocks the Na^+ channel [8–12]. Trimethoprim is a weak base (Table 1) and our data (Figs. 1 and 2, Table 2) suggest that, as in the case of amiloride, only protonated trimethoprim blocks Na^+ channels.

We have previously shown that amiloride blockade of the highly selective Na^+ channel in primary cultured rabbit CCD principal cells is also enhanced by membrane hyperpolarization. This observation would be predicted from the model for voltage-dependent amiloride block of the epithelial Na^+ conducting pore reviewed by Palmer [10]. Accentuating the cell negative potential draws the positively charged amiloride molecule into the channel pore, and retards its dissociation and return to the mucosal solution. In A6 cells, single channel studies by Eaton and Marunaka [9] measured amiloride blocking on- and off-rates that were compatible with an amiloride binding site within the pore, sensing 15% of the membrane voltage. The trimethoprim-induced block of epithelial Na^+ channels is also voltage dependent, providing evidence that it is the protonated, or charged species of trimethoprim which inhibits Na^+ channels.

Studies in vivo

The excretion of K^+ is a function of two factors, the flow rate in the CCD and the $[K^+]$ achieved in the luminal fluid of the terminal CCD [20]. To raise this luminal $[K^+]$, one needs an electrochemical driving force which is generated by the electrogenic reabsorption of Na^+ in this nephron segment. Electrogenic reabsorption, in turn, depends on a relatively more rapid reabsorption of Na^+ ions than Cl^- ions [21]. Hence the finding of a low TTKG or a low $[K^+]_{CCD}$ after administration of trimethoprim suggests that there was less electrogenic reabsorption of Na^+ in the CCD. This, together with the demonstration of inhibition of the Na^+ channel by trimethoprim in A6 cells (Figs. 1 and 2, Table

2) plus the obvious renal Na^+ wasting in patients [1] are all consistent with relatively slower reabsorption of Na^+ rather than relatively faster reabsorption of Cl^- [20] as the most important component of the pathophysiology. Since all rats in our study received a bolus of saline to expand their ECF volume and create a natriuresis, this model was not an ideal one to reveal the natriuretic actions of trimethoprim.

The hypothesis that was tested *in vivo* was that the cationic form of trimethoprim was the biologically active form of the drug. Given that its pK_a was in the range of pH of the urine that is seen *in vivo* (Table 1), the effect of a bicarbonate diuresis was studied. An alkaline urine produced by the administration of acetazolamide prevented a trimethoprim-induced low TTKG, and it increased renal K^+ excretion (Table 3).

Bicarbonaturia may also have three other effects to augment the rate of excretion of K^+. First, an increase in flow rate in the CCD due to an enhanced delivery of electrolytes might contribute to the increase in K^+ excretion observed in animals receiving trimethoprim and acetazolamide. Indeed, there was a higher rate of excretion of $Na^+ + K^+$ in rats treated with trimethoprim + acetazolamide as compared to trimethoprim alone (Table 3). Nevertheless, this augmented kaliuresis was accompanied by a much higher urine K^+ concentration and TTKG. Second, a relatively high concentration of bicarbonate in the CCD may inhibit Cl^- reabsorption, and thereby enhance K^+ secretion via a more negative intraluminal negative voltage as hypothesized by Velasquez, Wright and Good [22]. If this mechanism were operating to an important extent in the animals treated with trimethoprim and acetazolamide, one would expect enhanced Na^+-wasting compared to that seen with acetazolamide alone, since the trimethoprim would limit Na^+ reabsorption, and the Na^+ would then be excreted together with the non-reabsorbed Cl^-. In fact, Na^+ excretion rates were comparable in these two groups, suggesting that diminished Cl^- reabsorption due to enhanced HCO_3^- delivery to the CCD was not a major mechanism of enhanced K^+ excretion in acetazolamide-treated rats. Third, it has been suggested that the bicarbonate anion, when delivered to the CCD, is a nonreabsorbable anion, and thereby directly enhances K^+ secretion in this segment of the nephron [22]. We do not believe this was a major mechanism for K^+ secretion because nonresorbable anions augmented K excretion only when the concentration of Cl in the lumen was < 15 mmol/liter [22].

In summary, the evidence from the *in vivo* studies suggests that acetazolamide-induced bicarbonaturia causes trimethoprim to be converted from its protonated to its neutral form, and thereby prevents its Na^+ channel blocking activity which in turn abrogates the trimethoprim-induced diminished K^+ secretion. Other factors, such as a higher urine flow rate and reduced Cl^- reabsorption may also contribute, but evidence for these mechanisms was not found in our study.

Clinical implications

Patients with AIDS may be at greater risk of developing hyperkalemia than are other patients receiving trimethoprim [1, 2, 6, 13, 23, 24]. Either a higher intake or a lower output of K^+ could be responsible for their tendency to hyperkalemia. Since there is unlikely to be a greater dietary intake of K^+ in these patients who are often cachectic, the likeliest mechanism is a low excretion of K^+. K^+ excretion depends on the flow rate and $[K^+]_{CCD}$ in the terminal CCD. When ADH acts, the osmolality of luminal fluid in the terminal CCD should equal that in plasma [25]. At this fixed osmolality, flow rate is directly proportional to osmole excretion rate [26]. Since normal subjects have urea as their principal urine osmole, a low urea excretion rate could lead to a low flow rate in the CCD. Indeed, a low osmole (that is, urea) excretion rate may contribute to a lower rate of K excretion in patients with AIDS who are treated with trimethoprim [27].

A second mechanism for impaired renal K^+ excretion is that due to a very low $[K^+]$ in the luminal fluid of the CCD. In the context of the low K^+ excretion observed in these patients, a low open-probability of existing Na^+ channels due to hypoaldosteronism should first be ruled out [1]. More specific causes of a low concentration of K^+ in the lumen of the CCD related to trimethoprim would include either a higher dose of trimethoprim and/or a less alkaline luminal fluid. Factors that might lead to lower distal delivery of bicarbonate could include an acid ash diet, more protein catabolism due to sepsis (leading to greater production of H^+ ions [28]), more reabsorption of bicarbonate in proximal tubule cells (high angiotensin II associated with high renin levels caused by extracellular fluid volume contraction) and/or a lower pH in luminal fluid of the CCD because of aldosterone stimulated H^+ secretion in the CCD.

Concluding remarks

Since patients with AIDS who have PCP may best be treated by continuing their antimicrobial therapy (including trimethoprim), the aim should be to minimize renal side-effects of trimethoprim. To this end, a high intake of NaCl can combat the salt-wasting effects of the drug, and may even augment the kaliuresis by an increased urine flow rate. Nevertheless, there is the potential for giving too much or too little NaCl to accomplish these aims. From the results reported in this manuscript, we suggest that alkalinizing the urine with acetazolamide (250 mg) is a rational additional therapeutic measure to augment the excretion of K^+. Enough $NaHCO_3$ should be given to avoid metabolic acidosis and sufficient NaCl to defend the extracellular fluid volume. Careful monitoring of extracellular fluid volume, acid-base balance, urine pH and the plasma K^+ concentration will be important. Finally, the effects of acetazolamide on trimethoprim-induced inhibition of renal K^+ secretion should not be viewed as a unique phenomenon. Other epithelial Na^+ channel inhibitors (that is, triamterene; Table 1) with pK_as in the range of pH values that exist in the CCD urinary space may be less efficacious when urine pH values are higher with acetazolamide. As amiloride and pentamidine have higher pK_a values (Table 1), their effects on renal K^+ secretion should be insensitive to acetazolamide.

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